Laboratory evaluation of lactic acid on attraction of *Culex* spp. (Diptera: Culicidae)

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ABSTRACT: The role of lactic acid was evaluated for attraction of *Culex nigripalpus*, *Culex quinquefasciatus*, *Culex tarsalis,* and *Aedes aegypti* in the laboratory using a dual-port olfactometer. When lactic acid was combined with chicken odor, attraction was increased for *Cx. quinquefasciatus* compared to chicken odor alone but not for *Cx. nigripalpus*, *Cx. tarsalis,* and *Ae. aegypti*. Lactic acid combined with hand odor did not change attraction of *Cx. tarsalis* and *Ae. aegypti* but decreased attraction of *Cx. nigripalpus* and *Cx. quinquefasciatus*. The addition of lactic acid to CO_2 increased attraction of *Ae. aegypti* and *Cx. quinquefasciatus* but reduced attraction of *Cx. nigripalpus* and *Cx. tarsalis*. Use of commercial lactic acid baits with CO_2 resulted in a similar trend except for *Cx. nigripalpus* which showed no difference. A blend of lactic acid, acetone, and dimethyl disulfide was attractive to *Ae. aegypti* (63.4%) but elicited low responses by all *Culex* spp. (1.3-26.8%). Addition of the blend to CO_2 increased attraction of *Ae. aegypti* and *Cx. quinquefasciatus* but reduced attraction of *Cx. nigripalpus* and *Cx. tarsalis*. The mixture of compounds plus CO_2 was as attractive as a hand for *Cx. quinquefasciatus*, *Cx. tarsalis,* and *Ae. aegypti*. ***Journal of Vector Ecology* 35 (2): 318-324. 2010.**

Keyword Index: Culex, *Aedes*, mosquitoes, blood meal, surveillance, ecology.

INTRODUCTION

Host-finding by mosquitoes is a critical component of survival for most species and much research has focused on the cues used for host location. Of the many cues involved, host odor is generally considered the most important. Although mosquitoes feed on a broad range of hosts, humans have received the most attention. Considerable research has been conducted on odors produced by humans that elicit attraction in mosquitoes, with emphasis on compounds from sweat and skin (Smith et al. 1970, Eiras and Jepson 1991, Geier et al. 1996, Braks et al. 1999, 2001, Bernier et al. 1999, 2000, Constanini et al. 2001, Qiu et al. 2004, Okumu et al. 2010). Responses to these odors appear to be enhanced by CO_2, which is a universally present emission from vertebrates. Carbon dioxide is responsible for increased flight activity, attraction in some species, and sensitization of mosquitoes to host odors (Gillies 1980, Clements 1999, Dekker et al. 2005). The attractant activity of human sweat was initially associated with lactic acid (Acree et al. 1968). Smith et al. (1970) reported the enhanced attraction of mosquitoes to sweat in conjunction with CO_2 and concluded that other components of human odor may be involved as well. Subsequently, the role of lactic acid as a component of host attraction for *Aedes aegypti* (L.) and *Anopheles gambiae* Giles has been examined in detail (Acree et al. 1968, Schreck et al. 1990, Steib et al. 2001, Braks et al. 2001, Dekker et al. 2002, Bernier et al. 2003) and appears to be responsible for synergism with other compounds (Smallegange et al. 2005).

Other human-associated odors important in host location include acetone for attraction of *Ae. aegypti, An. gambiae, Anopheles stephensi* Liston (Takken et al. 1997, Bernier et al. 2003), dimethyl disulfide and dichloromethane for *Ae. aegypti* (Bernier et al. 2003), ammonia for *Ae. aegypti* (Geier et al. 1999) and *An. gambiae* (Braks et al. 2001), and other aliphatic carboxylic acids for *An. gambiae* (Constantini et al. 2001, Smallegange et al. 2009, Smallegange et al. 2010) and *Culex quinquefasciatus* Say (Puri et al. 2006). Electrophysiological and olfactometer responses to several carboxylic acids, alcohols, and aldehydes associated with human skin have been reported recently for *Culex quinquefasciatus* Say (Puri et al. 2006). While these compounds are most associated with humans in these studies, they are generally not unique to humans and may play a role in attraction to other hosts.

Previous studies associated high levels of lactic acid on human skin with strong attraction of anthropophilic species such as *An. gambiae* (Acree et al. 1968, Smith et al. 1970, Dekker et al. 2002) and *Ae. aegypti* (Acree et al. 1968, Smith et al. 1970). In contrast, birds, which are fed on readily by many *Culex* spp., may have relatively low levels of lactic acid in their emanations (Dekker et al. 2005). Lactic acid has been used to experimentally manipulate the attraction of anthropophilic species and, with the addition of lactic acid to host odor, results in increased attraction by *An. gambiae* (Dekker et al. 2002) and *Ae. aegypti* (Steib et al. 2001). In addition to its role as a host attractant, lactic acid also appears to contribute to host specificity for tsetse. The application of lactic acid onto an ox reduced attraction and feeding of the zoophilic species, *Glossina morsitans morsitans* Westw. and *Glossina pallidipes* Aust. (Vale 1979). Relatively little is known about the role of lactic acid and other human-associated compounds on attraction of *Culex* mosquitoes. The objective of this study was to determine if lactic acid and other human-associated chemicals influence attraction of several *Culex* species that differ in their attraction to humans and to compare their responses to *Aedes aegypti*.

MATERIALS AND METHODS

Mosquitoes

All species for this study were reared in the laboratory using methods described by Gerberg et al. (1994). Species in this study included: *Culex quinquefasciatus* (Gainesville, FL, established 1995), *Culex nigripalpus* Theobald (Vero Beach, FL, established 1999), *Culex tarsalis* Coquillett (Coachella Valley, CA, established 2001), and *Ae. aegypti* (Orlando, FL, established 1952). All of these *Culex* spp. feed readily on chickens yet differ in their attraction to humans (Allan et al. 2006a). Adults were maintained in screen cages with 5% sugar solutions provided continuously. Cages were held at 27-29° C and 70-85% RH under a photoperiod of 14:10 (L:D) with the onset of darkness at 20:00 for *Ae. aegypti* and at 11:00 for *Culex* spp. For bioassays, unfed 7- to 14-day-old female mosquitoes were used.

Olfactometer

Mosquito responses were evaluated in a triple-cage dual-port olfactometer (Posey et al. 1998) which consisted of a large clear acrylic chamber leading to two circular ports upwind of the chamber. Three chambers with corresponding test ports were stacked on each other with only one chamber used for assays at a time. External air was charcoal-filtered, humidified and warmed (27 $\pm$ 1° C, 60 $\pm$ 2% RH) and flowed through the ports at 28 $\pm$ 1 cm/s. Air flow through the ports was initiated by opening a door and at the end of a test, the door was closed, trapping mosquitoes in the ports where they could be counted. During a test, mosquitoes in the test chamber could follow an upwind air current to the treatment test port, to the control test port or remain in the chamber. Each chamber was loaded with 50-70 females collected from stock cages into release chambers using a draw box (Posey and Schreck 1981) that selectively collected active and responsive females. Mosquitoes were allowed to acclimate for ~1 h before testing. Responses were calculated as the percentage of total mosquitoes tested that were trapped in the treatment port or the control port. Treatments and controls were randomly assigned to the left or right ports. All materials placed in the treatment or control ports for testing were handled with gloves to avoid contamination with skin compounds. Each day, mosquitoes from each stock cage used were tested for responsiveness using a hand or CO_2 (5 ml/min) in preliminary olfactometer assays. If responses were below a pre-set criterion, assays were not conducted. A test consisted of placing treatment and control materials in the respective ports, opening the door to allow air flow over the materials into the test chamber, and closing the door at the end of the test. Assays consisted of 6 to 12 replicates and tests lasted for 10 min. Assays with *Ae. aegypti* were conducted under high light conditions (2,220-2,400 lux) between 10:00 and 15:00. Assays with *Culex* were conducted under low light conditions (100-150 lux) within 4 h after the onset of scotophase.

Assays

Tests were initially conducted comparing mosquito responses to a chicken (*Gallus gallus domesticus* L.) or human hand alone or in conjunction with L-lactic acid. For these tests, a treatment (chicken, hand, lactic acid, or combination of lactic acid and chicken or lactic acid and hand) was presented in one port and the other port was a blank control. To determine if lactic acid would negatively affect attraction to a chicken, a study was conducted using lactic acid, a chicken, or lactic acid in combination with a chicken as treatments. An unrestrained chicken was placed in an acrylic box (30.5 x 17.8 x 15.2 cm) with an average flow of 5 cm/s of air filtered, humidified, and warmed (Allan et al. 2006a). The chicken was allowed to settle (~5 min) before the test was initiated. Chickens were handled with gloves to reduce contamination with skin oils. Attraction to a human hand was evaluated by placing a hand through the iris diaphragm of the olfactometer into an olfactometer port. Hands were not washed within an hour of the test and contact with laboratory chemicals avoided. Care was taken so that hands did not contact the interior sides of the olfactometer to avoid contamination with skin compounds. Due to inherent differences in mosquito attraction to people (Steib et al. 2001, Qiu et al. 2006), only one individual was involved in the hand assays. Treatments using the combination of a hand or a chicken with lactic acid were tested as above except that 1 g of lactic acid α(technical grade, 98% purity, Sigma-Aldrich Chemical, St. Louis, MO) was placed in a watch glass (5 cm diameter) downwind of the hand or chicken in the treatment port.

The effect of lactic acid on attraction to *Culex* spp. was further examined by examining responses to CO_2 alone, lactic acid alone, and lactic acid in combination with CO_2 to determine if responses to CO_2 (5 ml/min) increased in the presence of lactic acid. For these tests, evaluations were made using technical grade lactic acid or commercial formulations of slow-release lactic acid. These products included Lurex™ (American Biophysics Corporation, North Frenchtown, RI) that releases ~0.23 g/day from a gel matrix (K. McKenzie, personal communication) and Insectagator™ (ICA, Trinova, Forest Park, GA) that consists of 5% lactic acid (w/w) in a powder. Treatments consisted of 1 g of lactic acid, one Lurex lure, or 1 gm Insectagator powder. Emission of lactic acid from the Insectagator formulation was 9.36 ± 0.04 mg/10 min trial compared to an estimated 1.59 μg/10 min from the Lurex bait and 1.38 ± 0.45 mg for technical grade lactic acid..

Attraction of these *Culex* spp. and *Ae. aegypti* females was evaluated using a lactic acid-containing mixture of three human-derived compounds previously shown to be effective in eliciting strong attraction of *Ae. aegypti* in the absence of CO_2 (Bernier et al. 2001, Silva et al. 2005). The mixture consisted of 480 ml acetone, 0.96 g lactic acid, and 10 ml of dimethyl disulfide, with 500 μl of the mixture applied to a watch glass immediately prior to each test. Mosquito responses were examined to the mixture alone, to CO_2, and to a combination of CO_2 and the mixture. Additionally, a human hand was added to the comparison to determine if these attractants were as attractive to *Culex* as a hand.

Attraction to the individual volatile compounds present in the lactic acid mixture was compared among the three *Culex* species. These included: acetone (98%), dimethyl disulfide (> 99.5%) (Sigma-Aldrich Chemical, St. Louis, MO) and lactic acid. Individual test compounds were placed in the treatment port of the olfactometer in vial caps (9 mm i.d. x 9 mm height) and compared against untreated vial caps in the control port. Compounds differed in volatility and volumes of treatments consisted of lactic acid (1 g), dimethyl disulfide (200 µl), and acetone (1 ml) so that compounds would be present for the entire assay duration. Emission rates of compounds based on reduced weights of three samples placed in the air flow of the olfactometer over a 10 min period were determined for dimethyl disulfide (273.15 ± 5.55 mg), acetone (0.65 ± 0.01 mg), and lactic acid (1.38 ± 0.45 mg).

Statistics

Responses between treatments and controls and between treatments were compared in the olfactometer by a paired *t*-test ($P < 0.05$). Before analysis, data were arcsine transformed and pre-transformation means are presented in tables.

RESULTS

Addition of lactic acid to the air stream containing chicken odors did not significantly decrease attraction of any of the *Culex* spp. to the chicken (Table 1). Responses to the chicken and to the chicken + lactic acid treatments did not differ significantly for *Ae. aegypti*, ($t = 1.00$; df = 22; $P = 0.16$), *Cx. nigripalpus* ($t = 0.80$; df = 22; $P = 0.43$), or *Cx. tarsalis* ($t = 1.43$; df = 22; $P = 0.08$). Responses of *Cx. quinquefasciatus* showed a significant increase with the addition of lactic acid to the chicken odor ($t = 2.83$; df = 22; $P = 0.01$). Addition of lactic acid to the air stream containing a hand in the olfactometer also did not affect responses of *Ae. aegypti* ($t = 0.94$; df = 22; $P = 0.18$) or *Cx. tarsalis* ($t = 0.43$; df = 22; $P = 0.33$) (Table 1). Responses of both *Cx. nigripalpus* ($t = 0.43$; df = 22; $P = 0.33$) and *Cx. quinquefasciatus* ($t = 2.19$; df = 22; $P = 0.05$) to a hand were significantly lower when lactic acid was added to the air stream.

The addition of lactic acid, either technical grade or as a slow-release commercial formulation, affected responses to CO_2 (Table 2). Responses to lactic acid alone were low for all species, with greatest responses by *Ae. aegypti* (19.3%) and lower responses by all three *Culex* spp. (0.3 – 6.7%). Responses to CO_2 alone ranged from 19.1% (*Ae. aegypti*) to 50.9% (*Cx. tarsalis*). When lactic acid was combined with CO_2, responses significantly greater than to CO_2 alone were observed by both *Ae. aegypti* ($t = 8.76$; df = 22; $P < 0.0001$) and *Cx. quinquefasciatus* ($t = 1.74$; df = 22; $P = 0.05$). In contrast, responses to the combination of lactic acid and CO_2 were significantly lower than to CO_2 alone for *Cx. nigripalpus* ($t = 2.75$; df = 22; $P = 0.012$) and *Cx. tarsalis* ($t = 2.38$; df = 22; $P = 0.022$).

Commercial formulations of slow-release lactic acid (Lurex, Insectagator) alone attracted relatively few mosquitoes in the olfactometer (0.0 – 6.5%) (Table 2). Compared to CO_2 alone, the addition of the Lurex lure resulted in significant increases in attraction of *Ae. aegypti* ($t = -2.19$; df = 22; $P = 0.03$) and *Cx. quinquefasciatus* ($t = -2.81$; df = 22; $P = 0.005$) but a significant decrease in attraction of *Cx. tarsalis* ($t = 4.89$; df = 22; $P = 0.002$). Addition of the Lurex lure did not change attraction of *Cx. nigripalpus* ($t = 1.42$; df = 22; $P = 0.08$). Compared to CO_2 alone, the addition of the Insectagator lure resulted in increased attraction of *Ae. aegypti* ($t = -3.69$; df = 22; $P = 0.008$) and *Cx. quinquefasciatus* ($t = -4.20$; df = 22; $P < 0.0001$) but no difference in attraction of *Cx. nigripalpus* ($t = -1.32$; df = 22; $P = 0.11$). Response of *Cx. tarsalis* ($t = 9.96$; df = 22; $P < 0.0001$) decreased significantly with the addition of the lure.

Response to the lactic acid-containing mixture of compounds was high in *Ae. aegypti*, moderate in *Cx. quinquefasciatus* and *Cx. nigripalpus,* and low in *Cx. tarsalis* (Table 3). Attraction to CO_2 was increased significantly in the presence of the lactic acid mixture for *Ae. aegypti* (~four-fold) and *Cx. quinquefasciatus* (~two-fold). For *Cx. nigripalpus* and *Cx. tarsalis*, however, attraction was decreased about 40%. Addition of CO_2 to the lactic acid mixture increased attraction above that to the lactic acid mixture for *Ae. aegypti* and *Cx. quinquefasciatus,* decreased attraction of *Cx. nigripalpus,* with no change for attraction of *Cx. nigripalpus*.

The lactic acid mixture + CO_2 was as attractive as a

Table 1. Effect of lactic acid on attraction to a chicken or hand by *Culex* and *Ae. aegypti*.

Treatment	Mean (SE) % of mosquitoes in treatment port			
	Cx. nigripalpus	*Cx. quinquefasciatus*	*Cx. tarsalis*	*Ae. aegypti*
Chicken alone	85.3 (1.4)	85.4 (2.4)	77.8 (2.5)	96.1 (2.4)
Chicken with lactic acid	87.0 (3.0)	93.3 (1.2)*	72.7 (0.6)	91.5 (2.8)
Hand alone	38.7 (2.1)	55.0 (1.8)	8.6 (1.7)	95.2 (1.8)
Hand with lactic acid	21.1 (1.2)*	31.7 (3.4)*	7.2 (1.3)	92.6 (1.8)

*Within each column (species), the mean of the treatment (chicken or hand alone) with lactic acid is significantly different than to the corresponding treatment alone (*t*-test, $P < 0.05$) (N = 12).

hand for *Ae. aegypti* and *Cx. quinquefasciatus*, less than to the hand for *Cx. nigripalpus,* and greater than to a hand for *Cx. tarsalis* (Table 3). Attraction to the lactic acid mixture was greater than to lactic acid alone (Table 3) for *Cx. nigripalpus*, *Cx. quinquefasciatus,* and *Ae. aegypti* but not for *Cx. tarsalis*.

Responses of *Ae. aegypti* differed from those of the three *Culex* spp. for the individual human-associated chemicals present in the lactic acid mixture (Table 4). *Aedes aegypti* was the only species that was more attracted to acetone and lactic acid than the corresponding untreated controls ($P < 0.05$). Dimethyl disulfide elicited significant attraction in both *Ae. aegypti* and *Cx. quinquefasciatus* ($P < 0.05$).

DISCUSSION

Lactic acid, in conjunction with other compounds such as host odors or CO_2, influenced attraction in the three *Culex* species tested. In *Cx. quinquefasciatus*, lactic acid played a role similar to that played in *Ae. aegypti*, however, responses by *Cx. quinquefasciatus* were lower (6.7%) than those by *Ae. aegypti* (19.3%). For species such as *Cx. nigripalpus* and *Cx. tarsalis,* lactic acid was not an attractant (0.3-1.7%) and significantly reduced attraction to CO_2 and to a hand. Responses to the chicken, however, were not decreased with the addition of lactic acid for *Cx. nigripalpus* and *Cx. tarsalis,* possibly due to the high levels of attractants emitted from the chicken. Previously, Allan et al. (2006a) compared the same three *Culex* species and *Ae. aegypti* to a human hand and to a chicken. All species responded similarly to the chicken, whereas attraction to the hand was very strong for *Ae. aegypti*, moderately low for *Cx. quinquefasciatus,* and very low for *Cx. nigripalpus* and *Cx. tarsalis*. In our study, the response of *Cx. tarsalis* was not reduced with addition of lactic acid to a hand, however, the overall response to a hand was very low (<10%) and the lack of attraction to human odors is in agreement with previous research on this species (McIver 1968, Allan et al. 2006a). *Culex tarsalis*, however, does appear to be strongly attracted by CO_2 (McIver 1968, Allan et al. 2006b) and the addition of lactic acid, either alone or as in the mixture of compounds to an air stream containing CO_2, reduced attraction in our study. *Culex nigripalpus* is not considered to respond well to humans as hosts under field (Provost 1969) or laboratory conditions (Allan et al. 2006a). In our study, the addition of lactic acid to the air stream containing a hand or CO_2 lowered attraction responses of *Cx. nigripalpus*. The reduced responses by both *Cx. nigripalpus* and *Cx. tarsalis* in the presence of lactic acid indicates that this compound may be involved in the mediation of host selection for these species with lactic acid acting as a deterrent.

Lactic acid alone is generally not considered a good attractant of mosquitoes and it is the combination of this compound with CO_2 that results in a synergized attraction in *Ae. aegypti* (Acree et al. 1968, Smith et al. 1970, Geier et al. 1999) and *An. gambiae* (Dekker et al. 2002). In landing assays, lactic acid elicited a moderate response in *Cx. nigripalpus* but not in *Cx. quinquefasciatus* (Allan et al. 2006a). In the same olfactometer study, only responses of *Cx. quinquefasciatus* were significantly greater than to controls. In a recent study, Dekker et al. (2005) documented an instant sensitization in *Ae. aegypti* in the presence of CO_2. In the present study, the addition of lactic acid to CO_2 enhanced attraction of *Cx. quinquefasciatus* and *Ae. aegypti* compared to CO_2 alone. This same trend was observed with the commercial slow-release formulations of lactic acid and the lactic acid mixture. The mixture of lactic acid, acetone, and dimethyl disulfide previously reported effective for attraction of *Ae. aegypti* in the absence of CO_2 (Bernier et al. 2001) elicited greater attraction in *Cx. quinquefasciatus* than to lactic acid. This was possibly due to attraction to other mixture components such as dimethyl disulfide, which was also attractive to this species in the olfactometer. The lactic acid mixture did not elicit attraction in *Cx. nigripalpus* nor did any of the individual compounds tested.

Commercial formulations of lactic acid appeared to release lactic acid with mosquito responses similar to those to technical grade lactic acid. Responses of mosquitoes to lures in conjunction with CO_2 followed a similar pattern as the study with lactic acid, with increased responses by *Ae. aegypti* and *Cx. quinquefasciatus* and decreased responses by *Cx. tarsalis*. These results underscore the fact that attractants available commercially are not universally attractive to all mosquito species, and consideration needs to be made of the target species and their propensity for attraction to the compound(s) released from the lures.

Previous field trials have provided some indication that lactic acid may negatively impact trap collections of *Culex*. Kline et al. (1990) tested various attractants under field conditions and reported that the addition of lactic acid to CO_2-baited CDC traps increased *Cx. nigripalpus* collections nearly three-fold. Addition of lactic acid to CO_2-baited traps, however, appeared to reduce collections of other *Culex* spp. Hoel et al. (2007) reported that traps baited with lactic acid and CO_2 collected fewer *Cx. nigripalpus* than traps baited with CO_2 or octenol and CO_2. Stryker and Young (1970) increased collections of only *Ae. aegypti* when adding lactic acid to traps. The impact of adding lactic acid as an attractant to traps that target *Culex quinquefasciatus* or *Culex tarsalis* remains to be examined.

Several studies support a role for lactic acid in host preference. Dekker et al. (2002) measured lactic acid levels from the skin of a numerous hosts including humans and cattle and suggested that the lower levels of lactic acid on bovine skin (9.4 μg/ml) compared to human skin (151 μg/ml) were related to the lower attraction of *An. gambiae* to bovines compared to humans. Levels of lactic acid detected from skin were proportional to the preference of the anthropophilic species, *An. gambiae,* for a host species. The host with the least lactic acid was the chicken (<1.0 μg/ml) which was not fed upon by *An. gambiae*. The preferred host, humans, had the highest levels of lactic acid of all species examined and the less preferred host, cattle, had moderately low levels of lactic acid. By adding lactic acid to cattle skin, attraction of *An. gambiae* was increased. Geier et al. (1996) removed lactate from human samples and found

Table 2. Effect of technical grade and commercial lactic acid treatments on attraction to CO_2 (5 ml/min) of *Culex* and *Ae. aegypti*.

	Mean (SE) % of mosquitoes in treatment port			
Treatment	*Cx. nigripalpus*	*Cx. quinquefasciatus*	*Cx. tarsalis*	*Ae. aegypti*
CO_2	28.3 (1.9)	25.7 (3.5)	50.9 (4.8)	19.1 (2.5)
Technical grade lactic acid				
Alone	0.3 (0.2)	6.7 (0.7)	1.7 (0.6)	19.3 (2.9)
With CO_2	18.9 (2.5)*	34.7 (2.0)*	30.0 (3.1)*	73.2 (5.2)*
Commercial lactic acid product				
Lurex alone	0.7 (0.4)	6.5 (2.3)	0.6 (0.6)	2.7 (1.2)
Lurex + CO_2	21.6 (4.2)	45.4 (4.8)*	14.6 (0.8)*	53.2 (13.7)*
Insectagator alone	4.8 (1.9)	4.1 (0.8)	0.0 (0.0)	4.6 (1.2)
Insectagator + CO_2	34.1 (3.3)	57.6 (5.8)*	32.6 (2.8)*	57.6 (11.5)*

*Within each species column, the mean of the lactic acid treatment + CO_2 is significantly different than CO_2 alone (*t*-test, $P < 0.05$) (N = 12).

Table 3. Effect of a lactic acid mixture attractive to *Ae. aegypti* on attraction of *Culex* to CO_2.

	Mean (SE) % of mosquitoes in treatment port			
Treatment	*Cx. nigripalpus*	*Cx. quinquefasciatus*	*Cx. tarsalis*	*Ae. aegypti*
CO_2	28.1 (2.2)	35.1 (4.4)	55.2 (4.2)	17.1 (2.4)
Lactic acid mix	17.9 (2.3)	26.8 (4.9)	1.3 (1.3)	63.4 (4.4)
Lactic acid mix + CO_2	15.7 (1.2)	64.6 (2.9)	15.6 (4.8)	87.9 (1.5)
Hand	30.5 (4.3)	55.2 (5.7)	5.2 (1.5)	91.3 (3.2)
	Values of *t* for comparisons			
CO_2 vs LA mix + CO_2	3.58**	-3.90**	3.26**	23.33**
LA mix vs LA mix + CO_2	-0.72ns[1]	7.80**	-2.38*	5.35**
Hand vs LA mix + CO_2	-2.77*	1.10ns	1.6ns	-2.70ns
LA alone[2] vs LA mix	-7.04**	-5.60**	0.50ns	7.20**

[1]ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$ (N = 10-12)
[2]Comparison made with data reported in Table 2.

Table 4. Comparison of attraction of three species of *Culex* and *Ae. aegypti* to odors associated with humans.

	Mean (SE) % of mosquitoes in treatment port			
Compounds	*Cx. nigripalpus*	*Cx. quinquefasciatus*	*Cx. tarsalis*	*Ae. aegypti*
Acetone	1.3 (0.3)	1.3 (0.4)	0.6 (0.2)	28.8 (4.3)*
Dimethyl disulfide	1.3 (0.7)	24.8 (3.6)*	2.0 (2.0)	9.5 (1.7)*
Lactic acid	1.6 (0.3)	4.9 (1.3)	1.3 (0.8)	18.2 (2.9)*

*Treatment means were significantly greater than controls (*t*-test, $P \leq 0.05$) (N = 6).

that none of the other components which contribute to the attractiveness of human skin for *Ae. aegypti* were effective without the addition of lactic acid. Steib et al. (2001) also reported that increasing levels of lactic acid on human skin or animal odor samples increased attractiveness to *Ae. aegypti*. Similarly, Vale (1979) reduced collections and feeding of tsetse flies on their preferred host, cattle, by adding lactic acid to the skin.

The three *Culex* species in this study belong to two of the nine host-feeding categories of Tempelis (1975) based on blood meal analysis. One category includes *Cx. quinquefasciatus,* which feeds readily on birds and mammals, in proportions possibly based on host abundance. There appear to be geographic differences regarding *Cx. quinquefasciatus* feeding on humans (Tempelis 1975), with the population from Florida feeding mainly on birds. Another category included both *Cx. nigripalpus* and *Cx. tarsalis,* species which switch seasonally from almost exclusively feeding on birds in the spring to mammal-feeding in the fall. Although neither species are considered anthropophilic (Tempelis et al. 1965, Tempelis 1975, Day 2005), they are effective arboviral vectors (Day 2005, Vitek et al. 2008). In our study, *Cx. quinquefasciatus* responded similarly to the human-feeding *Ae. aegypti* (Tempelis 1975) in their positive response to lactic acid, which is present in high levels with humans and low levels with birds (chickens). In contrast, *Cx. nigripalpus* and *Cx. tarsalis* responded negatively to lactic acid. This information is important in the development of effective surveillance tools for these species. Additional research conducted on the identification and evaluation of other host-associated chemicals may provide the basis for effective attractants for *Culex*.

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